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Letter to the Editor

Regulation of *Drosophila* Neurogenesis by RNA:RNA Duplexes?

The proneural genes of the *achaete-scute* complex (AS-C; *achaete* [*ac*], *scute* [*sc*], and *lethal of scute* [*l'sc*]) and *atonal* (*ato*) encode basic helix-loop-helix (bHLH) transcriptional activators that initiate neurogenesis in *Drosophila* (Jarman et al., 1993; Modolell, 1997). The spatial pattern of their activity is refined by multiple levels of negative control: their transcription (at least in the case of *ac*) is directly repressed by the bHLH protein Hairy, while their regulatory activity is antagonized by direct protein–protein interaction with the inhibitory HLH protein Extramacrochaetae (Emc) (Modolell, 1997). Subsequently, during lateral inhibition, a Notch pathway–mediated cell–cell signaling process, proneural gene expression appears to be directly repressed by bHLH proteins encoded by several Notch-regulated genes of the *Enhancer of split* complex [E(spl)-C] (Ohsako et al., 1994; Van Doren et al., 1994; Jimenez and Ish-Horowicz, 1997). The novel non-bHLH protein encoded by the *m4* gene of the E(spl)-C (Klämbt et al., 1989), which is similarly activated by Notch signaling, may also be involved in regulation of the proneural genes; it is related to another small protein with an apparent role in lateral inhibition, Bearded (Brd) (Leviten and Posakony, 1996; Leviten et al., 1997).

The 3′ untranslated regions (3′ UTRs) of *Brd*, *hairy*, and many genes of the E(spl)-C contain a novel class of sequence motif, the GY box (GYB, **GUCUUCC**); *emc* contains the variant sequence GUUUUCC (Lai and Posakony, 1997; Leviten et al., 1997; Figure 1A). Recently, we have also recognized that the 3′ UTRs of three proneural genes include a second type of sequence element, the proneural box (PB, **AAUGGAAGACAAU**; Figure 1A). The full 13 nt PB is found once each in *ac*, *l'sc*, and *ato*, along with a second, variant version in both *l'sc* and *ato* (Figure 1A). The presence of these motifs in such distantly related paralogs as *hairy* and certain bHLH genes of the E(spl)-C (for the GYB), and *ato* and two

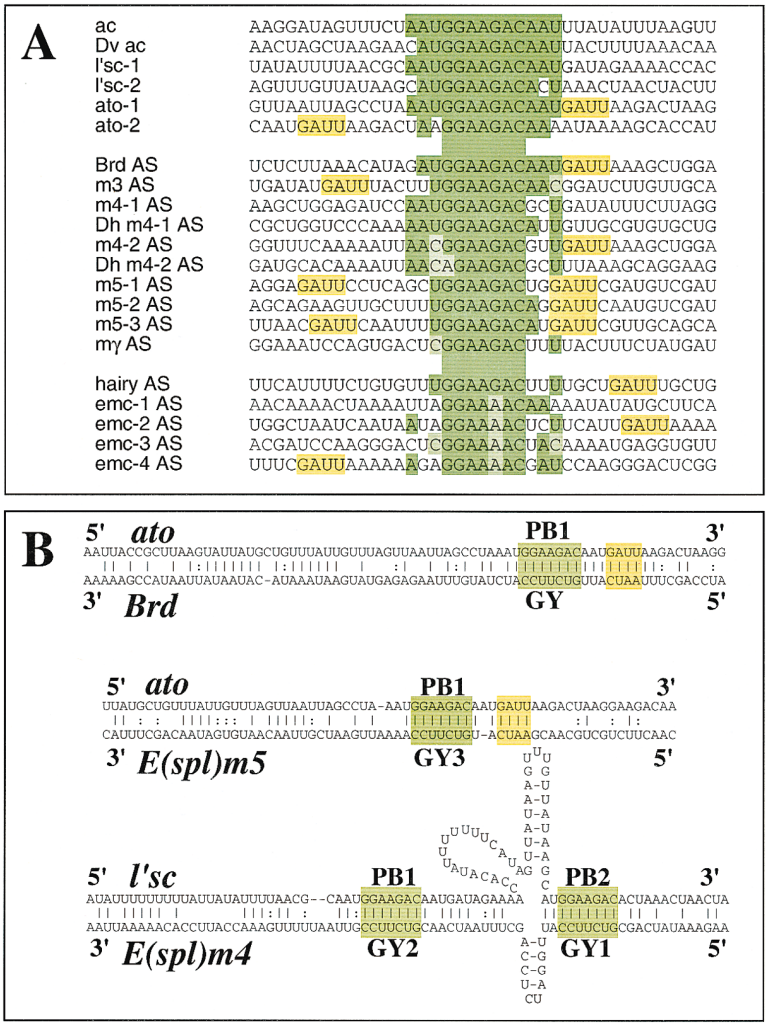

Figure 1. Complementary Sequence Motifs in the 3′ UTRs of Proneural Genes and Their Regulators

(A) Alignment of proneural boxes (PB) and GY boxes (GYB) in the 3′ UTRs of proneural genes and their regulators; the antisense (AS) strand of GYB-containing sequences is shown. Complementary nucleotides in the PB:GYB core are colored green; note that GAUU or its complement (yellow) flank many GYBs and the *ato* PBs, respectively. GenBank accession numbers for sequence data are as follows: *ac*, M17120; *l'sc*, X71806 and X12549; *ato*, L36646; *Brd*, AF016542; *m3*, M96165 and X67046; *m4*, X16551; *m5*, X16552; *m*γ, M96167 and X67049; *h*, X15904 and X15905; *emc*, M31902 and M32637.

(B) Extensive sequence complementarity often surrounds PB:GYB core duplexes (G:U basepairs are marked with colons).

genes of the AS-C (for the PB), indicates that both classes of sequence element are subject to strong selection. Furthermore, both the PB and the GYB are conserved in the orthologs of *ac* and *E(spl)m4* from the distantly related Drosophilids *D. virilis* and *D. hydei*, respectively (Figure 1A), though these 3′ UTRs are otherwise quite divergent from their *D. melanogaster* counterparts. These findings strongly suggest functional roles for both of these sequence elements.

Intriguingly, the central 7 nt of the PB and the GYB are exactly complementary, and are often located within extensive regions of RNA:RNA duplex predicted to form between PB- and GYB-containing 3′ UTRs (Figure 1B). Indeed, using in vitro assays we have observed RNA duplex formation between the *ato/Brd* and *ato/m4* 3′ UTR pairs that is PB- and GYB-dependent (our unpublished results). It is noteworthy that the predicted duplex interactions involving the GYB of *Brd* are significantly stronger than those involving the GYBs of the other transcripts. For example, *Brd* and *ato* are perfectly complementary over 18 contiguous nucleotides (Figure 1B). This difference in the degree of PB:GYB-associated complementarity is likely to have functional consequences.

In *C. elegans*, small antisense RNAs encoded by *lin-4* mediate translational repression of *lin-14* and *lin-28* transcripts by binding to complementary sequences in their 3′ UTRs (Slack and Ruvkun, 1997). We suggest that, in *Drosophila*, PB- and GYB-bearing transcripts may likewise participate in a regulatory mechanism mediated by RNA:RNA duplexes, but with the feature that both partners are mRNAs that also direct the synthesis of functionally interacting proteins. The opportunity to form such duplexes clearly exists, as transcripts from proneural genes and their regulators very frequently accumulate in coincident or overlapping patterns (Singson et al., 1994). Moreover, while 7 nt is the minimum length of complementarity between any PB and any GYB, the longest possible uninterrupted duplex between a given GYB-bearing transcript and a given proneural partner is almost always considerably longer (8–12 nt). It is worth noting that in a *lin-4/lin-14* duplex that has been shown to be sufficient for proper regulation in vivo, the longest region of uninterrupted complementarity is only 7 nt (Ha et al., 1996).

The formation of the postulated RNA duplexes may serve to regulate proneural gene function, consistent with the known roles of *hairy*, *emc*, and the bHLH genes of the E(spl)-C. This might explain occasional C-to-U transitions in the GYB sequence (in *emc* and *D. hydei m4*; Figure 1A); these variants retain complementarity with the PB due to G:U base-pairing. It is equally plausible that GYB-containing transcripts are regulated by duplex formation. A third very interesting possibility is that RNA:RNA duplexes formed between PB- and GYB-containing transcripts function to initiate a downstream regulatory activity affecting as-yet-unknown targets. Ample precedent exists establishing the *trans*-regulatory potency of double-stranded RNA (Jacobs and Langland, 1996; Nicholson, 1996; Williams, 1997). In any case, the apparent capacity of transcripts from the proneural genes and their regulators to form duplexes in their 3′ UTRs suggests further complexity in the already complex regulatory interactions that control *Drosophila* neurogenesis.

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References

Ha, I., Wightman, B., and Ruvkun, G. (1996). Genes Dev. *10*, 3041–3050.

Jacobs, B.L., and Langland, J.O. (1996). Virology *219*, 339–349.

Jarman, A.P., Grau, Y., Jan, L.Y., and Jan, Y.N. (1993). Cell *73*, 1307–1321.

Jimenez, G., and Ish-Horowicz, D. (1997). Mol. Cell. Biol. *17*, 4355–4362.

Klämbt, C., Knust, E., Tietze, K., and Campos-Ortega, J. (1989). EMBO J. *8*, 203–210.

Lai, E.C., and Posakony, J.W. (1997). Development *124*, 4847–4856.

Leviten, M.W., and Posakony, J.W. (1996). Dev. Biol. *176*, 264–283.

Leviten, M.W., Lai, E.C., and Posakony, J.W. (1997). Development *124*, 4039–4051.

Modolell, J. (1997). Perspect. Dev. Neurobiol. *4*, 285–296.

Nicholson, A.W. (1996). Progr. Nucleic Acid Res. Mol. Biol. *52*, 1–65.

Ohsako, S., Hyer, J., Panganiban, G., Oliver, I., and Caudy, M. (1994). Genes Dev. *8*, 2743–2755.

Singson, A., Leviten, M.W., Bang, A.G., Hua, X.H., and Posakony, J.W. (1994). Genes Dev. *8*, 2058–2071.

Slack, F., and Ruvkun, G. (1997). Annu. Rev. Genet. *31*, 611–634.

Van Doren, M., Bailey, A., Esnayra, J., Ede, K., and Posakony, J. (1994). Genes Dev. *8*, 2729–2742.

Williams, B.R. (1997). Biochem. Soc. Trans. *25*, 509–513.